XVIII. ENTEROINTOXICATION—ITS CAUSES AND TREATMENT.

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The function of the intestinal flora and the relationship of the flora to such of its products as are absorbed by the organism have been matters of some controversy.

Two conflicting views have been held, viz.

(1) That the intestinal microbes are useful to the organism.

(2) That the intestinal microbes are harmful to the organism.

The first hypothesis is nothing more than a teleological conception without supporting evidence; the second is based on observations, especially as to the relationship between the intestinal flora and the absence of urinary indican in the breast-fed child.

The contention has hitherto been left undecided owing to the lack of crucial experiments which alone are satisfactory for the elucidation of biological problems.

The present contribution deals shortly with experiments performed with a view to demonstrating that indican and other products in urine are due to the activity of intestinal microbes.

To put this conclusion on an unshakable basis it is necessary to demonstrate that *in vivo* an intestinal flora containing predominantly indole-forming micro-organisms gives rise to indican in the urine, whereas by transforming at will such a flora into one containing in predominance non-indole forming micro-organisms, the indican and other products disappear from the urine.

The first stage in the investigation of the relationship between intestinal flora and urinary poisons is to find a method of transforming the flora. This was done by feeding white rats upon a food diet which included lactose [Distaso and Schiller, 1914]. After feeding for two or three days with this diet the faeces acquire an acid reaction and a yellow colour. The predominant microbe is just the same as in breast-fed infants, viz. the *B. bifidus communis* which never produces indole. We were led to adopt this method starting from the hypothesis that certain enzymes may be absent in adult rats which would result in a sugar passing untouched into the caecum, and so into the large intestine, whereby microbes of what one of us has called *the acetogenous group* (*B. bifidus, acidophilus*, etc.) would be able to grow [Distaso, 1911].

Under these conditions just as is the case in experiments *in vitro* the higher the percentage of sugar available for the intestinal microbes the more quickly the acetogenous flora gain the ascendency, and afterwards they are the only species which survive. We dismissed, therefore, the idea of introducing new microbes into the intestine because in each intestinal flora there are plenty of acid producers and non-indole forming organisms which are always ready to seize the first opportunity for displaying activity. Moreover we found to our astonishment that we were unable to acclimatise strange microbes to the environment of the intestinal flora even though the most favourable conditions were provided [Distaso and Schiller, 1914].

The discovery of this method opens up a wide field from the physiological and pathological standpoint. The rapid sensibility of the intestinal flora to such influence should certainly permit us to solve the question of the relationship between intestinal flora and the urinary poisons, and to acquire a more exact knowledge as regards the activity of the enzymes of the digestive canal in each animal species under normal and pathological conditions and assuredly supplies a more direct and trustworthy method of investigation than any optical method can.

At any rate one problem in connection with the nutrition of the breast-fed child seems to be explained in connection with this discovery. Certainly in this case a sugar is prepared in the breast of the mother, and this is not absorbed by the young organism, but passes untouched into the large intestine. Why the mother prepares in her breast something which is of no nutritive value to the suckling is one of the riddles which it is hard to understand if one carefully avoids teleological explanations.

Relationship between Indole and Microbes.

In this connection, the first point which can be demonstrated *in vitro* is that the indole, of which the indican is the oxidation product, is formed by the activity of the microbe alone. In fact if proteins are really split by sterile methods in the presence of intestinal juice, it will be observed that the products of the cleavage reach the tryptophan stage. If the same process is carried out in the presence of microbes, indole will be at once detected. The indole is, therefore, a product of the activity of the microbes, and is not formed by the enzymes of the digestion. This conclusion is supported by Nencki's classical experiments *in vivo*. He found that the products of digestion drawn off through an iliac fistula never contain a trace of indole, whereas it is easy to detect indole in the faeces where we know the microbes display all their activity. Thus *in vitro* as well as *in vivo* it has been demonstrated that indole is formed by the activity of the microbe.

We now proceed to give below some of our experiments which demonstrate that indican disappears from urine if we cause the indole forming flora to disappear, whereas it reappears as soon as the indole forming flora reappears.

We have, moreover, dealt with the question of the production of ethereal sulphates and skatoxyl and we believe there is evidence enough of the mechanism and source of their production.

The feeding experiments were made at intervals over a considerable period, under varying conditions with regard to diet and amount of lactose used. In all cases control feeding experiments were made at the same time as the lactose feeding tests, using groups of from one to three animals as available. The amount of food given (other than lactose) was not weighed, but the animals were of approximately equal size, and were supplied with the food in approximately equal amounts.

Putrefaction of the urine was inhibited by admixture with small amounts of suitable antiseptic.

It was necessary at times to continue the collection for a period of 48 hours or more, although, had the conditions permitted, it would have been more satisfactory to have been able to make analyses upon the freshly collected samples.

Analytical Results.

Indoxyl. The figures given under this term may be regarded as approximating to the indoxyl present, since the colouring matter was formed in the presence of an oxidising agent, and therefore indigo blue should be the chief product and its amount be approximately in proportion.

10 cc. of urine previously treated with basic lead acetate and filtered, were mixed with an equal volume of pure strong HCl and extracted with 2 cc. of chloroform, adding 1–2 drops of 1 in 10 H_2O_2.

A second extraction was made where any considerable development of indigo resulted. The intensity of colour was then matched against a standard tint on an arbitrary scale. No great degree of accuracy can be attained by the method and the figures are merely relative, but they serve for purposes of comparison.

Amyl Alcohol Extract.

The residual acid liquid was extracted once or twice with small amounts of amyl alcohol, and as with the chloroform extract the coloration obtained was recorded on an arbitrary scale.

To what extent these figures give a measure of the skatoxyl pigment is a matter of controversy; also the tints were not clearly defined and colorimetric readings were more indefinite than with the blue chloroform extracts.

Ethereal Sulphates.

These determinations as well as those of the mineral sulphate were made by the benzidine method as described by Rosenheim and Drummond. The figures are given in terms of milligrams SO_3 per animal per diem. They will

include not only the indoxyl sulphates but also any skatoxyl sulphates, phenol sulphates, etc. It will be found that the few determinations which could be made show some relationship (as is to be anticipated) to the figures for indoxyl.

We give below a few typical experiments for the sake of brevity.

Experiment I.

Control—Potato
Lactose—Potato + 2·5 g. lactose per animal.
From October 30 to November 16, 1916.

Test		Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
1916					
Nov. 3	Control ...	10·5	0·6	6·9	0·6
Nov. 6	,, ...	3·5	0·8	7·3	0·3
Nov. 10	,, ...	Nil	—	5·0	Nil
Nov. 12	,, ...	1·0	—	6·1	Nil
Nov. 16	,, ...	Nil	—	2·3	Nil
Nov. 5	Lactose 2·5 g.	Nil	0·1	—	—
Nov. 11	,, ...	Nil	—	2·7	Nil
Nov. 16	,, ...	Nil	—	1·5	Nil

Remarks. The continued feeding of the control animals with potato only shows a gradual reduction in the figures for indoxyl and also for ethereal sulphates which after two weeks were practically nil.

Although the lactose feeding experiment shows the rapid disappearance of indoxyl and other products within a few days, the experiment is in our opinion inconclusive.

We have been obliged to select another diet which would give a greater and a persistent amount of indican, skatoxyl, ethereal sulphates, etc., in the control, a condition under which alone a crucial experiment would assume its full value.

Experiment 2.

Control—Banana.
Lactose—Banana + 2·5 g. lactose per animal.
From March 14 to March 30, 1917.

Test		Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
March 19	Control ...	Traces	2·0	—	—
March 22	,, ...	1·5	2·2	0·25	0·1
March 27	,, ...	2·4	0·4	0·3	0·1
March 30	,, ...	1·9	1·2	0·15	0·05
March 20	Lactose 2·5 g.	0·4	0·2	—	—
March 23	,, ...	Nil	0·2	0·15	Nil
March 27	,, ...	Nil	1·0	0·1	Nil
March 30	,, ...	Nil	0·8	—	—

Remarks. In the control feeding the figures for ethereal sulphates (also mineral sulphates) were exceptionally low throughout, but they are capable of comparison.

Lactose feeding gave negative results within a few days for both indoxyl and ethereal sulphates, but the amyl alcohol extract continues to be appreciable in the lactose-fed animals.

Experiment 3.

Control—Expt. 2 Lactose animals now fed with banana.
Lactose—Expt. 2 Control animals now fed with banana + 2·5 g. lactose per animal.
From March 30 to April 23, 1917.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
April 2	Control ...	Nil	1·6	0·12	Nil
April 4	„ ...	1·1	0·5	0·14	Nil
April 6	„ ...	7·0	0·7	0·09	0·03
April 7	„ ...	2·0	0·5	—	—
April 10	„ ...	1·0	1·5	—	—
April 17	„ ...	1·2	0·6	—	—
April 19	„ ...	3·0	1·0	—	—
April 23	„ ...	2·0	1·0	—	—
April 3	Lactose 2·5 g.	Nil	0·3	—	—
April 7	„	Nil	0·3	—	—
April 10	„	Nil	Prac. Nil	—	—
April 13	„	Nil	„	—	—
April 17	Banana only	Nil	„	—	—
April 20	„	0·5	0·5	—	—
April 23	„	0·5	0·5	—	—

Remarks. Feeding reversed. On feeding the original control animals with lactose, negative results for indoxyl were obtained within a few days. The original lactose-fed animals (now banana only) showed a gradual re-appearance of indoxyl.

The same phenomena were to be observed in this experiment as in the previous one; with the appearance of indican, the flora showed chiefly coliform and allied micro-organisms, whereas with its disappearance the acetogenous group partially re-appeared.

We attribute the persistence of the figures for amyl alcohol extract to the fact that it is almost impossible to mix thoroughly the lactose with banana. Further, the transformation of the intestinal flora thereafter was not complete, smears made from films actually showing an appreciable proportion of *B. coliformis*.

Experiment 4.

May 24 to June 8.
Control—Bread for two days previously, then bread and egg.
Lactose—Bread for two days previously, then bread and egg* + lactose: 4 g. to June 2; 5 g. to June 6; 8 g. to June 8 per animal.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
May 24	Control ...	14·0	Small amount	—	—
May 27	,, ...	22·0	Fair amount	—	—
May 29	,, ...	32·0	4·0	—	—
May 31	,, ...	16·0	1·6	12·5	1·2
June 2	,, ...	24·0	1·5	—	—
June 4	,, ...	28·0	2·0	—	—
June 6	,, ...	32·0	2·0	—	—
June 8	,, ...	22·0	2·2	24·1	2·8
May 24	Lactose 4·0 g.	12·0	Small amount	—	—
May 27	,,	10·0	Fair amount	—	—
May 29	,,	6·0	0·8	—	—
May 31	,,	3·0	0·4	4·6	0·25
June 2	,,	2·4	< 1·0	—	—
June 4	,, 5·0 g.	< 0·05	0·5	3·4	0·5
June 6	,,	Prac. nil	1·0	4·9	0·5
June 8	,,	0·8	0·4	4·3	0·3

* This was prepared as follows: an egg was beaten and diluted with 4 times its volume of water. The bread was soaked in this emulsion.

Remarks. The control feeding gave very high figures for indoxyl. The lactose feeding experiment shows a gradual reduction in indoxyl, ultimately to nil, with a large reduction in ethereal sulphates.

The experiment was stopped at this stage, because the rats began to refuse their food and, as will be seen later, this antipathy towards lactose has been an adverse factor to cope with, which has required special conditions of feeding.

Experiment 5.

June 14 to July 16.
Control—Bread and egg; fed on bread for six days previously.
Lactose—Bread and egg + 8 g. lactose per animal up to June 22.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
June 14	Control ...	3·0	—	—	—
June 16	,, ...	13·0	Fair amount	—	—
June 18	,, ...	30·0	,,	—	—
June 20	,, ...	17·5	—	11·3	0·7
June 22	,, ...	12·0	1·8	—	—
June 29	Bread only	18·0	Small amount	—	—
June 30	,, ...	13·0	Fair amount	—	—
July 11	,, ...	17·5	,,	—	—
July 16	,, ...	16·0	,,	—	—
June 14	,, ...	7·0	—	—	—
June 16	Lactose 8 g.	1·5	Small amount	—	—
June 18	,, ...	0·2	,,	—	—
June 20	,, ...	0·3	—	0·2	Practically nil
June 22	,, ...	0·4	Nil	—	—
June 27	Bread only	1·6	Traces	—	—
June 30	,, ...	1·6	< 0·8	—	—
July 11	,, ...	4·5	Small amount	—	—
July 16	,, ...	10·0	Apprec. amount	—	—

Remarks. The control feeding showed high figures for indoxyl and these were still maintained on a reversed diet of bread only. In the lactose feeding a quick reduction was obtained in both ethereal sulphates and indoxyl to practically nil. The indoxyl gradually re-appeared after stopping feeding with lactose, reaching the normal amounts in about three weeks.

Experiments 4 *and* 5.

Although in these experiments we have been unable to reduce the amounts of indican, amyl alcohol extract and ethereal sulphate to nil, this partial failure is, in our opinion, due to certain adverse circumstances, *viz.*

(1) The diet was really very severe.

(2) We have been obliged to use old animals which owing to long experience had become unsuitable for use.

(3) The rats have a distaste for lactose.

In order to avoid a condition of starvation in this and in other experiments they were discontinued whenever these adverse factors were becoming acute.

Experiment 6.

July 23 to August 18.

Control—Fed on bread three days previously, then bread and egg.

Lactose—Fed on bread three days previously, then bread and egg to July 26, then 8 g. lactose, then 5 g., then 10 g. lactose per animal.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
July 23	Control ...	28·0	Apprec. amount	—	—
July 25	,, ...	20·0	1·5	13·4	1·0
July 28	,, ...	19·0	1·7	—	—
July 31	,, ...	33·0	3·2	—	—
Aug. 3	,, ...	16·0	1·1	14·3	0·7
Aug. 6	,, ...	27·0	1·5	—	—
Aug. 10	,, ...	Large amount	1·1	—	—
Aug. 13	,, ...	21·0	1·8	16·4	1·1
Aug. 15	,, ...	10·0	Small amount	—	—
Aug. 18	,, ...	9·0	Fair amount	—	—
July 23	Bread ...	32·0	Fair amount	—	—
July 25	Bread and egg	19·0	1·9	13·2	1·0
July 28	Bread and egg + Lactose 8 g.	< 1·0	0·3	—	—
Aug. 1	,,	0·4	0·2	—	—
Aug. 3	,, 5 g.	0·6	0·5	6·8	Nil
Aug. 6	,,	0·4	Practically nil	—	—
Aug. 10	,,	Trace only < 0·05	0·2	—	—
Aug. 13	,, 10 g	0·3	Practically nil	0·2	Nil
Aug. 15	,,	2·0	Traces	—	—
Aug. 18	,,	4·2	Apprec. amount < control	—	—

Remarks. The control feeding showed high figures for indoxyl which were reduced towards the end of the period. With lactose feeding the amounts of both indoxyl and ethereal sulphates were quickly reduced to almost nil and finally also the mineral sulphates, but afterwards the indoxyl began to reappear.

This is an illustrative experiment in which the results obtained may appear to indicate a failure on our part to produce systematically and always the disappearance of urinary poisons. But the flora in this case suggests the explanation. Here it is to be noted that there is an enormous fall in urinary poisons after three days of lactose feeding, but at the same time the flora was far from showing the homogeneity seen in other cases. This condition had continued for 13 days, when we decided to increase the amount of lactose. Now the rats took little food, it being almost entirely rejected—a condition of starvation in which all the classical symptoms ensued and which led to a definite increase in the urinary poisons. The flora on the other hand again returned to the condition of the control flora, viz. predominance of *B. coliformis*.

Experiment 7.

September 17 to October 7, 1917.
Control—Bread and egg.
Lactose—Bread and egg + 8 g. lactose per animal.

	Test	Indoxyl	Amyl Alcohol Extract
Sept. 17	Control Bread ...	6·0	1·2
Sept. 19	„ Bread + egg	6·5	1·9
Sept. 21	„ „	8·0	2·6
Sept. 24	„ „	12·0	2·3
Sept. 26	„ „	10·0	1·8
Sept. 29	„ „	7·2	1·3
Oct. 1	„ „	14·5	1·2
Oct. 4	„ „	13·0	1·3
Oct. 7	„ „	13·0	0·5
Sept. 17	...	5·5	1·4
Sept. 19	Lactose 8 g. ...	2·0	0·5
Sept. 21	„ ...	1·0	0·3
Sept. 24	„ ...	2·2	0·4
Sept. 26	„ ...	1·6	0·4
Sept. 29	„ ...	1·2	0·6
Oct. 1	„ ...	1·1	0·7
Oct. 4	„ ...	1·5	1·0
Oct. 7	„ ...	0·5	0·5

Remarks. Old rats used many times. The control feeding gave indoxyl in fair amount, but less than in earlier experiments. With the lactose feeding a slow gradual reduction in amount of indoxyl is to be noted. A certain amount of indican, although small, persisted to the end of the experiment, and an observation of interest is that the flora always contained a certain number of Gram-negative bacilli (*B. coliformis*).

As previously stated, the rats were very fastidious in choosing their food, selecting the crumbs and rejecting lactose so far as possible, and of course without lactose introduced into the intestine the experiment will fail.

It is very difficult to secure a thorough admixture of lactose with bread and therefore for later experiments we chose cooked potatoes to which an emulsion of egg was added.

Experiment 8.

Control—Potato and egg.
Lactose—Potato and egg + 8 g. lactose per animal.
Nov. 14 to Nov. 27, 1917.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
Nov. 14	Control	8·5	—	—	—
Nov. 20	„	8·5	Trace only	—	—
Nov. 24	„	5·0	Practically nil	—	—
Nov. 27	„	8·0	„	10·4	0·6
Nov. 14	Before Lactose ...	8·4	—	—	—
Nov. 20	+ Lactose 8 g. ...	0·2	—	—	—
Nov. 24	„ ...	Nil	0·3	—	—
Nov. 27	„ ...	Nil	Practically nil	7·3	Nil

Remarks. The control feeding showed indoxyl and ethereal sulphates in fair amount, less than in the earlier feeding with bread and egg, but the amounts were persistent throughout the experiment. With the lactose feeding a rapid elimination of indoxyl occurred with the practical elimination of ethereal sulphates within ten days.

Experiment 9.

Control—Potato and egg.
Lactose—Potato and egg + 8 g. lactose per animal.
December 18, 1917, to January 7, 1918.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
Dec. 20	Control	8·5	Practically nil	—	—
Dec. 22	„	5·0	0·6	—	—
Dec. 24	„	4·5	0·7	8·4	0·9
Dec. 25	„	Fair amount	Small amount	—	—
Dec. 31	„	4·5	„	—	—
Jan. 2	„	7·4	„	—	—
Jan. 4	„	7·8	1·3	18·0	0·8
Jan. 7	„	8·0	Nil	14·5	1·9
Dec. 20	Lactose 8 g. ...	0·2	Practically nil	—	—
Dec. 22	„	Nil	Nil	—	—
Dec. 24	„	Nil	Nil	—	—
Dec. 28	„	Nil	Nil	—	—
Dec. 31	„	Nil	Nil	—	—
Jan. 2	„	Nil	Nil	—	—
Jan. 4	„	Nil	Nil	8·3	Nil
Jan. 7	„	Nil	Nil	12·3	Nil

Remarks. The control feeding results were similar to those in Experiment 8, whilst the lactose experiment also gave results practically identical with the previous Experiment 8. With this diet we have, therefore, reached the ideal experimental conditions for proving our assertion and in this and the following experiment it will be seen that with these conditions realised the experiments will give consistently the same results.

Experiment 10.

Control—Potato and egg.
Lactose—Potato and egg + 8 g. lactose* per animal.
March 12 to 26, 1918.

	Test	Indoxyl	Amyl Alcohol Extract	Mineral Sulphate mg. SO_3	Ethereal Sulphate mg. SO_3
March 18	Control	3·4	1·2	—	—
March 19	,, ...	8·4	2·0	24·0	2·2
*March 20	,, ...	5·4	Small amount	—	—
*March 22	,, ...	9·6	,,	15·9	2·45
*March 23	,, ...	12·8	,,	—	—
March 25	,, ...	19·0	,,	16·3	2·0
March 26	,, ...	17·0	Yellowish	—	—
March 18	Lactose 8 g. ...	Nil	Nil	—	—
March 19	,, ...	Nil	Nil	—	—
March 20	,, ...	Nil	Nil	—	—
March 22	,, ...	Nil	Nil	—	—
March 23	,, ...	Faint trace	—	—	—
		< 0·1	Practically nil		
March 25	,, ...	Nil	Nil	7·7	Nil
March 26	,, ...	Nil	Nil	13·2	Nil

* Since under the conditions of the experiments some direct admixture of lactose with the urine was unavoidable, an amount of lactose approximately equal to that given in the food was in these control tests added to the containing vessels, giving a direct admixture of the urine with lactose with no marked fluctuations in the results obtained.

Remarks. In the control feeding the amounts of indoxyl and ethereal sulphates reached a higher figure than with previous experiments. The lactose experiment showed a quick elimination of indoxyl and also of ethereal sulphates.

Conclusions.

We have been able to show that the presence of indoxyl, skatoxyl and ethereal sulphates in the urine, corresponds with a flora composed of microbes which *in vitro* give indole and skatole.

On the other hand if such a flora be transformed into one which does not produce either indole or skatole, then indoxyl, skatoxyl and ethereal sulphates are absent from the urine.

The group of microbes which produces these poisons is that of *B. coli-formis* which play a great rôle in many intestinal disorders and states of ill-health, and to produce disintoxication the intestinal flora must be transformed so as to consist of non-indole producers.

It is interesting to note that throughout our experiments the total mineral sulphates are lower in the non-intoxicated animals, and this observation should open up an experimental field of considerable importance in relation to the metabolism of the mineral constituents of the animal body.

We admit that the amount of mineral matter in the food used will influence the excretion of the mineral sulphates, but nevertheless our experiments show throughout a marked decrease of the total mineral sulphates with lactose feeding.

This effect we attribute to the fact that in intestinal stasis and in cases where intestinal fermentation is abnormal, the food is far more broken down by the action of the microbes and therefore more absorption of the mineral products takes place, with greater excretion in the urine.

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